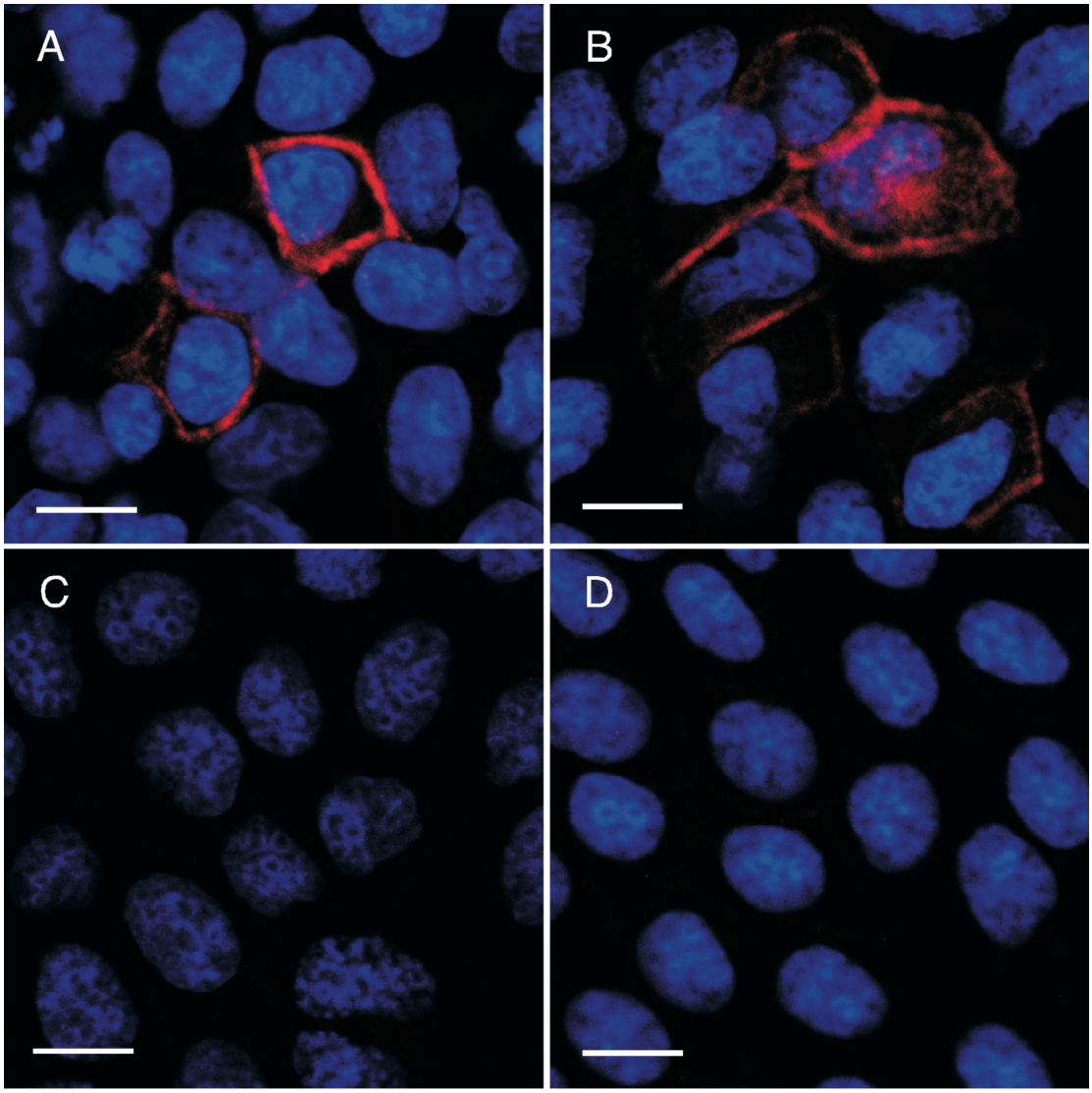
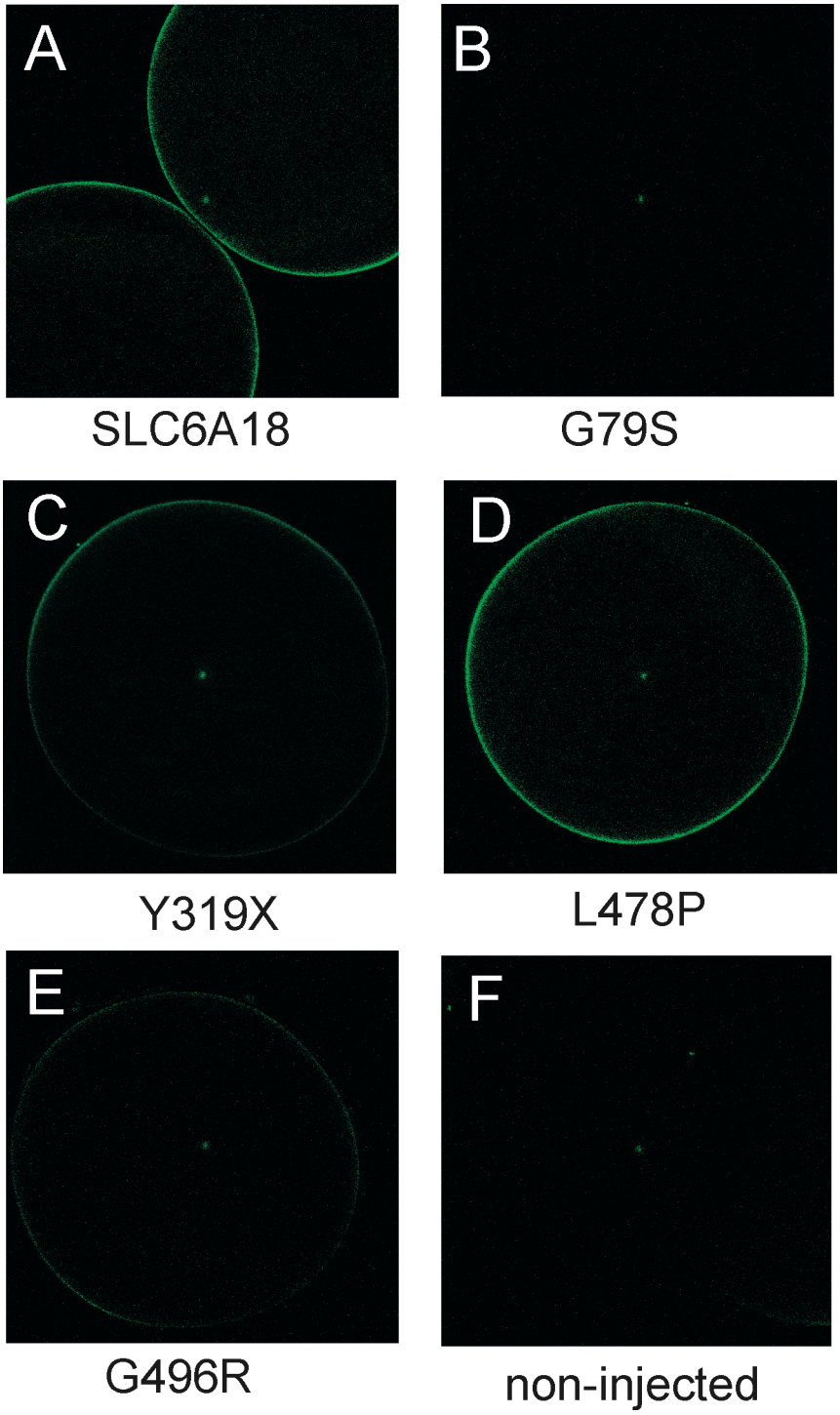
Supplemental Data

Supplemental Figures



Supplemental Figure 1 Effect of the *SLC36A2* IVS1+1G>A mutation on protein expression. MDCKII cells transfected with the *SLC36A2* minigenes (Figure 5) were stained for surface expression of the C-terminal epitope FLAG by immunofluorescence: (A) *SLC36A2* full-length; (B) *SLC36A2* wildtype IVS1+1G; (C) *SLC36A2* mutant IVS1+1A; (D) *SLC6A20* (no FLAG) negative control. Confocal images were magnified at 60X. Scale bars represent 10 µM. Staining for the c-myc epitope showed similar patterns of expression as FLAG by immunofluorescence (not shown). The IVS1+1A mutant eliminated expression of *SLC36A2*.



Supplemental Figure 2 Surface expression of SLC6A18 wildtype and mutants in *Xenopus laevis* oocytes. Oocytes were injected with cRNA encoding a wildtype or mutant eGFP-SLC6A18 fusion. After incubation for four days, surface expression was analyzed by confocal microscopy. Surface expression of SLC6A18 L478P (D) was similar to that observed in the wildtype (A). Expression of mutants G79S (B) and G496R (E), by contrast, was abolished and appeared similar to non-injected oocytes (F). The truncated protein Y319X showed significantly reduced expression (C).

Supplemental Tables

Subject	Age (yr)	Ref. Range	Hydroxyproline	Proline	Glycine
1.1	40	A	0, 0, 0	0, 0, **1**	124, 167, 192
1.2	37	A	0, 0, 0	0, 0, 0	132, **306**, 172
1.3	5	B	**220,** 0**, 35**	**180, 221, 33**	**822, 1053, 576**
1.4	5	B	**250, 72, 52**	**205, 55, 148**	**1121, 757, 669**
2.1	40	A	0, 0	0, 0	**494, 360**
2.2	40	A	0, 0	0, 0	**483, 354**
2.3	4	B	**117,** 0	**357,** 0	**1660, 1193**
2.4	3	B	**140,** 0	**178,** 0	**1577, 1416**
3.1	43	A	0, 0, 0	0, 0, **1**	104, **279**, 165
3.2	43	A	0, 0, 0	0, 0, 0	**413, 403, 274**
3.3	3	B	**120,** 0**, 49**	**149, 658, 148**	**1965, 854, 805**
4.1	54	C	0	0	**177**
4.2	47	C	0	0	**231**
4.3	23	C	0	0	132
4.4[e]	19	C	**150, 40**	**259, 163**	**1747, 1609**
5.1	DEC	N/A	N/A	N/A	N/A
5.2	47	C	0, 0	0, 0	94, 117
5.3	14	C	0, 0	0, 0	**174,** 160
5.4	14	C	0, 0	0, 0	51, 78
6.1	N/A	N/A	N/A	N/A	N/A
6.2	44	C	0	0	**333**
6.3	18	C	0	0	104
6.4	14	C	0	0	**377**
7.1	59	C	0	0	**300**
7.2	50	C	0, 0	0, 0	173, **199**

7.3	21	C	0	0	155
7.4	18	C	0	0	**287**
7.5	16	C	0	0	**632**
7.6	12	D	**227**	**1037**	**1025**
	adult	A	*0*	*0*	*0-198*
	infant	B	*0*	*0-3*	*0-379*
	adult	C	*0*	*0*	*43-173*
	infant	D	*0*	*0*	*64-165*

Supplemental Table 1 Analysis of urinary amino acids. Age indicates patient age at initial analysis in this study, N/A = not available, DEC = deceased. All amino acids were normalized to creatinine (µmol/mmol creatinine); *A, B*, indicate standard ranges used in the Quebec Mass Urinary Screening program, *C, D,* indicate standard ranges used in the New South Wales Newborn Screening program; *e*, Pregnant at time of sampling, some neutral amino acids were elevated above normal reference ranges (not shown).

Primer name	Forward Primer	Reverse Primer	Amplicon Size (bp)
SLC36A1ex1	CAGTTCCTATTCGCAGTCCC	CACCATCTACCCCAGTTTCC	591
SLC36A1ex2	TTTGCACTTTTCCATTTCCC	CCAATCTCTTCACTGCTGACC	446
SLC36A1ex3	TGTGACCTTGAGCAAACTGC	TCGAGCCATCTCAGTACATCC	525
SLC36A1ex4	TCACCTCACATCCCTTAGCC	CAGAAAAGAACGCAAAAATAACC	589
SLC36A1ex5	GGGTGAAAGATGGGAACTGG	GGCAACTGACCCAAACAGC	321
SLC36A1ex6	AAAAACACCTCCCCTTCTGG	ATGTGGCGGTTTCTCTATGC	357
SLC36A1ex7	CACACACCTGAGCCTTTCC	CCTCAGCCTCCCAAATAGC	551
SLC36A1ex8	TACCTTGGGCTTGTCTTTCC	CTGGGATTATAGGCGTGTGC	600
SLC36A1ex9	GCAGCAGGAGGACAGAGC	GGTGGGCTAGAGTGAAGTGG	564
SLC36A1ex10	GGACCATTTTGCACTCTTGC	ACCTCCACAGCATTTCATACC	500
SLC36A1ex11	GTGGGACGCCAGTTATTCC	CTCAGAGCCTGGACCATACC	549
SLC36A2ex1	CCTTCCTCTCCTCCTCTTCC	GCCTTTACTTCTGCCTCAGC	555
SLC36A2ex2	CTTCTCACCTCCACACACCC	CAGAGCCGGAACTCAAACC	304
SLC36A2ex3	GCCCAGTTTTCCAAGTATGC	TATGCCCAATTTCCAGAGG	653
SLC36A2ex4	CTCTGCCCCTGTGTTCTTTC	CAGCCCTTCTTTCCTTCATC	628
SLC36A2ex5	TTGTTGATTGTGGTGGAAGG	CCGGATAAAGGGTATTGTGG	430
SLC36A2ex6	CTCATCATCTCCCCCTTTTG	GTTTTCGCCTGCCTTTTG	590
SLC36A2ex7	TGGAGTTTCGCTCTTTCACC	GGCCACCTCAATTCAAACC	633
SLC36A2ex8	CCCTGCTTTCAGTTCTTTGG	TGCCCTTCCCTTCTCTCC	562
SLC36A2ex9	TGACCCTTTCCATTGTTTCC	TTGCCTTGGTTCTTGAGTAGC	646

SLC36A2ex10	GAGATGGGATTTCACTATGTTGG	AAAGTCGGGTGCTGGTAGG	533
SLC6A18ex1	GAGAAGGAGCAGAGGACGTG	TGAGGTCCCAGAAGACTGC	585
SLC6A18ex2	ACGCTTAGACCCCAAGTCCT	GCTTCTCACACACACCCAAG	588
SLC6A18ex3	AAGCTGGTGTTGCCATTCAC	GCTGCTCTGATGCTGGACAC	387
SLC6A18ex4	AGAAGTGAAAGGGCACCTCA	ACAAAGCGAACACAGGTCAA	392
SLC6A18ex5	AGGAGAGAGGCAAGTGGAGG	TGAAATGTGTGTGCTGGAGG	536
SLC6A18ex6	ACGTTCTGGAACAGTCAGGG	CTGAGATCAGGAAGTGCTCG	259
SLC6A18ex7	CCCTCCTCACTTCCTCCC	TCCTCCTCTGTGTCTGTCTCC	501
SLC6A18ex8	GTCCACACGATCCCAACAG	CCTGGCAACCTGAGACAAAG	546
SLC6A18ex9	TGTGGTGGAGTGTGTGTGTG	CTCAGCCTTTCCCTGAACTC	391
SLC6A18ex10	TGGGAAGCCGAGAGAAGTC	AAGGTCCACCCACTCCAAG	501
SLC6A18ex11	CTTGGAGTGGGTGGACCT	CTGTTCACATCCCTGGCTTC	389
SLC6A18ex12	TTTCCATTCCCATCCAAGTC	CCCTGCGAGACCTCCCTA	550
SLC6A19ex1	AGATCTGCTGACGACTGGCTCCGT	CTTCTCCTCTCCCCTCCGTGCTT	474
SLC6A19ex2	CAGCTCTGTTCCCAAGTGGTGGGC	CCTTTGTTGCACCAGAAACAGACAGGG	402
SLC6A19ex3	ATCAGCTGCATGATCCCG	GGTCTTTTCTTTCGTGCTGTC	460
SLC6A19ex4	TGTGTGCCTGTGTTCATGTG	CAGTGAGCCTAAGGGCAAAG	489
SLC6A19ex5	ACCCTTCCAGCATGCCTC	GGACTGGTCGAGCAGCAG	378
SLC6A19ex6	CTGGCCTGTCCTGACCAC	GACTCAGCTCACATGGACCC	395
SLC6A19ex7/8	GTGGCTCAGCCTCTCACTCC	CAGAGCAGACAGTGGGAGC	647
SLC6A19ex9	CACTTTGGGCCTAAAAGACAA	ACAGAGCTGTTCACACGCC	486
SLC6A19ex10	GGCATGTGAGCAGCTCTGT	CAGGTCTACACCAGCCTCTG	473

SLC6A19ex11	CACAGAGGGGTCTGCTTGAG	GAGTGTGAGTGTGTGTGCCC	465
SLC6A19ex12	GAGTTGGGAGGTAGGGGAAA	ACACGAGCATACACTCACGC	482
SLC6A20ex1	TCGATAAATGACAGGTGAGTGG	TGTGCCGTTCTGTAACTTGG	584
SLC6A20ex2	ATGAATGGATGGTTGGATGG	CAGAGAAGAGAAGGAGGGTGG	679
SLC6A20ex3	GGTGAGGAGGGAGGTTGG	GCTGTCTTGCTGGATTGAGG	695
SLC6A20ex4	CCTGACAAACTCTCCAACTCCCAG	CATCTGTGCCCTCTGCCCTAAC	554
SLC6A20ex5	CACTTCCCCAGCCCTAACC	TCTCCACCTTTCCCTCTTCC	376
SLC6A20ex6	ATGGCTGCGTGCTGTAGG	GCAACTGGTGGTGGTGTAGG	505
SLC6A20ex7	AACAGACTCCCTTTCCTCACC	TTTACAGACCTCGCCTATCTCC	368
SLC6A20ex8	GTAAGTTGGGGCAGGAGAGG	CCCTGTGATGTGGAGAATGG	677
SLC6A20ex9	GCACACACCTTCAGAGAGTCC	GCATGGAATAGAGGCAGAGC	506
SLC6A20ex10	CCAAGCTCAGACATCCTTTACC	CCCCACTGAAGCACATACC	494
SLC6A20ex11	AAGAAGGTGAGGGCAGTTAGG	GAGGAGGCAGGTGACAGG	554

Supplemental Table 2 Primers designed to amplify across exon/intron boundaries in *SLC36A1*, *SLC36A2*, *SLC6A18*, *SLC6A19* and *SLC6A20* for subsequent sequencing reactions.

Application	Forward Primer	Reverse Primer	Comments
SLC36A2 IVS1+1G>A exon 1	CCTTCCTCTCCTCCTCTTCC	GCCTTTACTTCTGCCTCAGC	RFLP for IVS+1G>A, product 554 bp digest with *HpyCH4IV*, normal: 385bp, 169 bp, mutant: 554bp
SLC36A2 G87V exon 3	GCCCAGTTTTCCAAGTATGC	TTATGCCCAATTTCCAGAGG	RFLP for G87V, product 653 bp digest with *ApaI* , normal: 330 bp, 323 bp, mutant: 653 bp
SLC6A20 T199M	GTTGAAGGGAGGGTGGAAGG	GCAATAGGGCAGTGACGACG	Allele specific PCR R: T199, product 318 bp
		GCAATAGGGCAGTGACGACA	R: M199, note mismatch at -3 in both reverse primers
SLC6A18 Y319X	CTTGTGTGCGTTTCTCCTTTGG	CCTGTCCAGGCAGTGCACG	Allele specific PCR R: Y319, product 477bp
		CCTGTCCAGGCAGTGCACC	R: X319, note mismatch at -3 in both reverse primers
SLC6A18 G496R	TGGCCTTTCTCGAGGTTGTG	GTCCCAGAGGAGCACTCG	84 bp
	VIC-TCGTTTATGTTTATGGAATGA-MGB		G496, Taqman Probe, VIC-labelled
	6FAM-TCGTTTATGTTTATAGAATGA-MGB		R496, Taqman Probe, 6FAM-labelled
SLC6A19 IVS7-4G>A exon 8	TCAGCCTCAATCTGACTCG	TCGGAAGTGAAATCTCGTGG	Allele specific PCR F: SLC6A19 IVS7-4G, product 387bp
	TCAGCCTCAATCTGACTCA		F: SLC6A19 IVS7-4A
SLC6A20 cDNA	CCACCATGGAGAAAGCGCGGCCGCTGT	AAGCTTGATGGGCTGAGCACT	F: Kozak sequence, R: *HindIII* restriction site, 1892 bp
SLC36A2 cDNA	GGAATTCCACCATGTCTGTGACA	GGAATTCTGGGCAACAAGACAGA	F: *EcoRI* site, Kozak sequence, R: *EcoRI* site, 1573 bp
SLC6A18 cDNA	AGCCACCATGGCTCATGCCCCAGA	AAGGCCGGCTTCAGCGCATGTC	F: Kozak sequence, 1902 bp
SLC36A2 G87V	GGCATCCTGATGGTCCCACTCAGTC	CAGACTGAGTGGGACCATCAGGATGCC	GGG to GGT, Gly to Val, mutagenesis primers

	TG		
SLC6A20 T199M	AGGTGGTGTATTTCATGGCGTCACT GCCCT	AGGGCAGTGACGCCATGAAATACACC ACCT	ACG to ATG, Thr to Met, mutagenesis primers
SLC6A18 G79S	GTCGAGCTCGCCATCAGCCAGCGG CTGCGGAAG	CTTCCGCAGCCGCTGGCTGATGGC GAGCTCGAC	GGC to AGC, Gly to Ser, mutagenesis primers
SLC6A18 Y319X	GCAACTAATGACTAGGAGCACTGCC TGGAC	GTCCAGGCAGTGCTCCTAGTCATTAGT TGC	TAC to TAG, Tyr to TAG(stop), mutagenesis primers
SLC6A18 L478P	AATTTTGCCGCTTCCCCGAACCTGC TCATGTTG	CAACATGAGCAGGTTCGGGGAAGCGG CAAAATT	CTG to CCG, Leu to Pro, mutagenesis primers
SLC6A18 G496R	GTCGTTTATGTTTATCGAATGAAAC GGTTC	GAACCGTTTCATTCGATAAACATAAAC GAC	GGA to CGA, Gly to Arg, mutagenesis primers
EGFP transporter	CCTAATACGACTCACTATAGGGCCA CCATGGTGAGCAAG	TCAGTTATCTAGATCCGGTGGATCCCG GGG	F: contains T7 promoter sequence for *in vitro* transcription
IVS+1G>A RT-PCR	AGAACCCACTGCTTACTGGC	TGGGGTTGGCTTCTAGTCC	F: 1st round primer, R: primer X
		AAGGCCTGGAACACTGTTATG	R: primer Y
		AAGGCCTGGAACACTCTCAG	R: primer Z
IVS+1G>A RT-PCR semi-nested	GCTTATCTCGGAGGAGGACC		F: primer W (semi-nested primer, 2nd round), used with reverse primers X, Y or Z.

Supplemental Table 3 Primers and probes used for cloning, RT-PCR and allele frequencies.